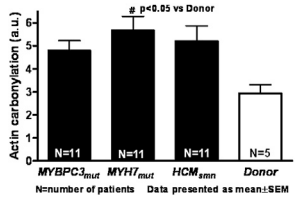
and chemiluminescence using appropriate antibodies. Actin-carbonylation was higher in HCM patients compared to donors. It was highest in MYH7mut(Figure).

**Conclusion:** Increased actin-carbonylation could partially underlie reduced force development in human HCM. Increased oxidative stress may contribute to impaired contractile function during disease development in sarcomere mutation-positive and mutation-negative HCM patients.




# Microtubules, Their Motors, and Associated Proteins II

**3925-Pos Board B653**

**Structural Kinetics of the Mitotic Kinesin Eg5**

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Members of the kinesin superfamily of molecular motors differ in several key structural domains, which probably allow these molecular motors to serve the different physiologies required of them. One of the most variable of these is a stem-loop motif referred to as L5. This loop is longest in the mitotic kinesin Eg5, and previous structural studies have shown that it can assume different conformations in different nucleotide states. However enzymatic domains often consist of a mixture of conformations whose distribution shifts in response to substrate binding or product release, and this information is not available from the "static" images that structural studies provide. We have addressed this issue in the case of Eg5 by attaching a fluorescent probe to L5 and examining its fluorescence, using both steady state and time-resolved methods. This reveals that L5 assumes an equilibrium mixture of three orientations that differ in their local environment and segmental mobility. Combining these studies with transient state kinetics demonstrates that there is a major shift in this distribution during transitions that interconvert weak and strong microtubule binding states. Finally, in conjunction with cryoEM reconstructions of Eg5:microtubule complexes, these fluorescence studies suggest a model in which L5 regulates both nucleotide and microtubule binding through a set of reversible interactions with helix α3. We propose that these features facilitate the production of sustained opposing force by Eg5, which underlies its role in supporting formation of a bipolar spindle in mitosis.

**3926-Pos Board B654**

**Structural Basis for the Assembly of Kinesin-5 into Bipolar Anti-Parallel Tetramers**

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Cell division is driven by organized mitotic spindles and requires diverse groups of molecular microtubule-based kinesin motor proteins. Kinesin-5 motors form a unique and conserved class of tetrameric bipolar motor proteins, consisting of dual motor domain sets oriented at opposite ends of a central rod-like structure. The bipolar organization is critical for kinesin-5 to crosslink and slide adjacent anti-parallel microtubules in opposite directions required for spindle elongation in anaphase. Here we present the atomic structure of Drosphila kinesin-5, KLF61F, bipolar assembly (BASS) domain, which explains how four molecules assemble to form a bipolar functional motor. Instead of the predicted dual traversing parallel coiled-coils, the BASS structure reveals a novel 26nm long antiparallel 4-helical bundle filament, where the basic assembly unit is an anti-parallel coiled-coil that originate from opposite ends of the bipolar structure, and folds onto a second unit in an anti-parallel manner. A striated pattern of hydrophobic and hydrophilic pockets precisely oriented monomers within the BASS tetramer to form this bipolar arrangement. Strikingly, the BASS N-termini undergo a helical exchange from anti-parallel bundle in the center to form homo-dimeric parallel helical coiled-coils at the poles. These parallel BASS N-termini are 100 degrees rotated compared to N-termini emerging from the opposite pole, providing a physical explanation for the known kinesin-5 preference for sliding anti-parallel versus parallel microtubules. We have generated a structural model for the kinesin-5 tetramer using the BASS structures and all electron microscopy that explains key features of the kinesin-5-driven sliding filament mechanism.

**3927-Pos Board B655**

**Kinesin-5 Motility is Regulated by the Residue Chemistry of Loop-5**

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Kinesin-5 drives separation of microtubules and organizes the mitotic spindle via its motor domains. More than one component within Kinesin-5 has been implicated in its motile ability; these are Loop-5, the necklinker and the cover neck. In all studies, measured changes in structure, or their implied dynamic motion, were deemed key to the role of these components. Unaddressed is whether these protein components chemically direct mechanical output. Herein, we show that specific side-chain chemistry of Loop-5 must be conserved for productive Kinesin-5 motility. Six substitutions of Loop-5 residues were created in the Eg5 dimer by mutagenesis, and their microtubule-gliding velocities were measured. Mutant Eg5 gliding velocities ranged from nearly equivalent to wildtype to a complete loss of motility. There were key differences between the mutations in the N- versus C-terminus of Loop-5. Substitutions near the N-terminus retained the ability to glide microtubules; alteration of gliding velocity paralleled changes in microtubule-stimulated catalytic rates. In contrast, nonconservative substitutions near the C-terminus of Loop-5 bound microtubules in rigor despite having robust ATPase activity. Collectively, these results suggest the integrity of the active site remains intact and communication between the active site and microtubule site is not compromised. Mechanical output is challenged, however. Therefore, we conclude that side-chain interactions of these C-terminal residues with the surrounding protein matrix are required for the terminal step in Eg5 mechanotransduction. Given our kinetic data, we speculate that aberrant interactions may result in changes in force and/or coordination of the two motor domains in the dimer, rendering Eg5 incapable of motility. This work is funded by the support of the National Institutes of Health (R01 GM097350 S.K.;P20GM103424 and 5G12RR026260 T.H.) and the LSU School of Graduate Studies (R.B.).

**3928-Pos Board B656**

**A Chimeric Kinesin-5 Motor Tracks Plus-Ends of Microtubules**

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Kinesin-5 is necessary for bipolar spindle formation during mitosis. Besides its unique homotetrameric configuration, determined by the coiled-coil domain, the kinesin-5 motor domain also possesses specific properties suitable for its spindle organizing function. To study the walking properties of functional kinesin-5 dimers, we fused the kinesin-5 head and neck linker domain to the coiled-coil rod of kinesin-1. We report that a chimeric kinesin-5 head/kinesin-1 rod construct with 14 aa neck linker tracks plus-ends of both growing and static (taxol-stabilized) microtubules with a mean residence time of roughly 7 seconds. The same construct having a 18 aa neck linker, as found in wild-type kinesin-5, also remained bound to the plus-end of static microtubules, but labeling at growing microtubule plus-ends was not observed. These phenomena explain previous reported end-to-end tethering of microtubules following antiparallel sliding by full length kinesin-5. This end-labeling presumably occurs because the motor walks to the end of the microtubule and is unable to take a subsequent step. We hypothesize that kinesin-5 remains bound at the plus-ends of microtubule by a single head in the ATP state and that hydrolysis and dissociation of this head requires binding of the tethered head to the next binding site on the microtubule. Computational modeling based on this hypothesis predicts that motor accumulation at the plus-ends of growing microtubules relies on motor processivity, consistent with experimental observations. Our studies reveal a property intrinsic to the kinesin-5 head domain that is distinct from transport motors such as kinesin-1 and kinesin-2.

**3929-Pos Board B657**

**Cut7-Driven Microtubule Sliding Reverses Direction Depending on Motor Density**

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Cut 7, the only kinesin-5 in S. pombe, is essential for mitosis. We have found that full length Cut7 slides microtubules (MTs) bidirectionally, with MT sliding